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THE BIOLOGICAL EFFECTS OF CRUDE AFLATOXIN B_1 ON MUSTARD (*BRASSICA JUNCEA*) VARIETIES

MANISH KUMAR & AHMAD MASOOD

Department of Botany, H. D. Jain College, Ara, Bihar, India

ABSTRACT

Three commonly cultivated varieties of mustard (Brassica juncea Hook. f and Thomas) viz, Pusa Bold, Samruddhi Gold and J.K. Suraj were treated with crude aflatoxin B_1, extracted from Aspergillus flavus with toxic potential of 0.9 ppm.

The effect on seed germination, seedling growth, chlorophyll and protein syntheses were recorded. An inhibition of 35.4 to 42.6% in seed germination, maximum being in case of Samruddhi Gold was found. Seedling growth comprising root and shoot lengths also showed a marked reduction. An inhibition of 32.9, 40.8 and 37.4% in root length and 41.5, 46.5 and 46.3% in shoot length were recorded in Pusa Bold, Samruddhi Gold and J.K. Suraj, respectively. Visual chlorosis was observed in the emerging leaves of all the varieties. A depletion of 30.5 to 42.3% in total chlorophyll was recorded in the varieties. A significant inhibition in protein biosynthesis was also noted. It was 15.9% in Pusa Bold, 22.8% in Samruddhi Gold and 26.8% in J.K. Suraj.

KEYWORDS: Aflatoxin B_1, Mustard & Biological Effect

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Original Article

INTRODUCTION

The food crops have been found to be frequently infected with aflatoxin, the toxic metabolite of *Aspergillus flavus*, resulting in significant economic burden on food and feed industries (Robens & Cardwell, 2005). Mustard (*Brassica juncea* Hook. f and Thomas) is an important oil-seed crop of Bihar. Surveys and studies during the past few years have shown that the crop is susceptible to *A. flavus* infestation and subsequent aflatoxin production [Sinha, et al. 1988, Bilgrami et al., 1991). Recently Masood & Kumar (2011) have also found mustard and other food crops to be heavily infected with aflatoxin producing isolates of *A. flavus.* While infecting the crops, the quality of seed is also deterioted by the fungus. The degraded seed with reduced nutrient contents may be the risk factor to consumers, because aflatoxin is acutely toxic and a known carcinogenic factor (IARC, 1993).

The present investigation aimed to study the important biological changes as brought out by the crude aflatoxin B_1 on three commonly cultivated varieties of mustard Viz; Pusa Bold, Samruddhi Gold and J.K. Suraj.

MATERIALS AND METHODS

The varieties of mustard (*Brassica juncea* Hook. f and Thomas) Viz; Pusa Bold, Samruddhi Gold and J.K. Suraj were obtained from the local government farm house of Bhojpur district headquartered at Ara. The crude aflatoxin B_1 was extracted from a toxigenic isolate of *Aspergillus flavus* using SMKY liquid medium (Diener & Davis, 1966). The isolate was collected from the mustard seed sample and had aflatoxin producing capacity of

about 0.9 ppm.

The seed samples were surface sterilized with 0.1% mercuric chloride solution for 2 minutes and subsequently washed with sterile distilled water. After proper washing the test samples were put in sterile distilled water for 1 hr. and subsequently soaked in freshly prepared aqueous aflatoxin B_1 extract for 20 hrs. For control soaking was done in distilled water.

A total of ten seeds each of treated and control lots were placed separately in Petri plates containing moistened blotting paper with thin layer of sterile water soaked cotton. Germination counts were recorded after an incubation of 72 hrs. A total of 3 replicates of 100 seeds of each experimental variety of mustard were taken.

The seedling growth was measured on the 7th day by recording the root and shoot lengths. The experiment was repeated three times with two replicates of 20 seeds each.

Chlorophyll content of newly emerged leaf was estimated by the method of Arnon (1949). Total chlorophyll was calculated by combining the values of Chla and b. The quantitative estimation of total protein was done by the method of Lowry et al. (1993).

RESULTS AND DISCUSSIONS

An inhibition of 42.6% in seed germination was recorded in Samruddhi Gold with the treatment of aflatoxin B_1. Other varietie had 41.5% (J.K. Suraj) and 35.4% (Pusa Bold) inhibition in the germination (Table-1). The seedling growth comprising root and shoot lengths also showed a marked reduction (Table-2). The suppression in root length was 32.9 to 40.8% in the three varieties. The shoot growth was found to be reduced upto 46.5% in Samruddhi Gold. The other varieties ware found to be affected more or less similarly showing 41.5% inhibition in Pusa Bold and 46.3% in J.K. Suraj.

The toxic effect of aflatoxin inhibiting the seed germination has been recorded by several investigators. Mehan & Chohan (1974) found complete inhibition in seed germination of *Phaseolus aureus* due to aflatoxin treatment. Other crops like sorghum (Tripathi & Misra, 1983), maize (Sinha & Kumari, 1989) & wheat (Sinha, 1991) were also investigated and a marked reduction in germination processes was found. In recent past Janardhan et al. (2011) found a vigorous reduction in the germination of seed of some pulses when soaked in the culture filtrates of toxic *A. flavus* strain. Aflatoxin B_1 which occurs mostly as internal seed contaminant by *A. flavus*, probably interferes the overall process of germination as well as seedling growth.

The effect of aflatoxin B_1 on chlorophyll and proteins, the two important biochemical parameters, was also evident. Visual chlorosis in emerging leaves was substantiated by a marked reduction in the contents of chlorophylls a and b. The total chlorophyll content was inhibited by 30.5, 31.4 and 42.3% in Pusa Bold, Samruddhi Gold and J.K. Suraj respectively (Figure 1).

Table 1: Effect of Crude Aflatoxin on Seed Germination in Mustard.

Variety	Aflatoxin Treatment	Percent Germination (S.F.)	Percent Inhibition in Germination	't' Defference Value
Pusa Bold	Control Treated	96 (1.13) 62 (2.80)	– 35.4	11.3
Samruddhi Gold	Control Treated	94 (1.37) 54 (2.88)	– 42.6	12.5
J.K. Suraj	Control Treated	94 (1.37) 55 (2.87)	– 41.5	12.3

Values are significant at 1% Level

Table 2: Effect of Crude Aflatoxin on Seedling Growth in Mustard

Variety	Aflatoxin Treatment	Root Length (cm)		Shoot Length (cm)	
		Mean ±S.D	% Inhibition	Mean ±S.D	% Inhibition
Pusa Bold	Control Treated	9.86 ±0.56 6.62 ±0.53	– 32.9	6.46 ±0.38 3.78 ±0.32	– 41.5
Samruddhi Gold	Control Treated	10.83 ±0.45 6.41 ±0.50	– 40.8	6.86 ±0.28 3.67 ±0.36	– 46.5
J.K. Suraj	Control Treated	10.23 ±0.34 6.40 ±0.46	– 37.4	6.91 ±0.35 3.71 ±0.34	– 46.3

Protein which is the building block of all the living organisms, was also found to be markedly affected by the toxin treatment. The inhibition was between 15.9 to 26.8% in the three varieties of mustard (Figure 2).

A depletion in chlorophyll and protein contents by the toxin treatment is a manifestion of interference of aflatoxin B_1 at the level of biosynthesis of those compounds. Chohan (1983) is of the opinion that mycotoxin checks the synthesis of chlorophyll by restricting the growth hormone induced synthesis of RNA, DNA and Protein in the leaf. Recently, a depletion of chlorophyll in some maize varieties treated with aflatoxin was also recorded (Deepavali & Nilima, 2012).

The protein contents which is inhibited to a marked level by aflatoxin B_1, reflects the probability of suppression of several biochemical processes. Some of which may affect the growth of the plant as well.

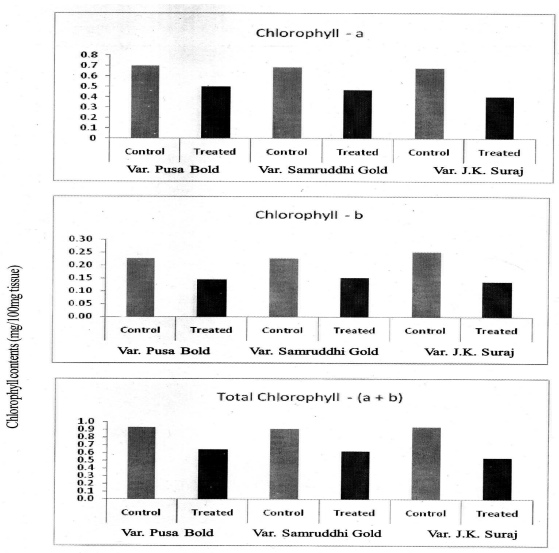

Figure 1: Effect of Crude Aflotoxin on Chloropfyll a, b and Total Chlorophyll Contents in Mustard Seedlings

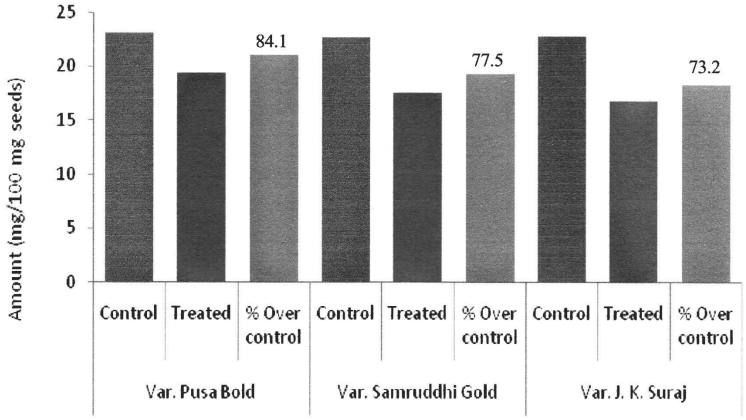

Figure 2: Effect of Crude Afloxation on Protein Contents in Germinating Mustard Seeds

CONCLUSIONS

The Contamination of food crop by fungi and accumulation of mycotoxins Particularly aflatoxins are serious agrigultural problem. As evident from the result, crude aflatoxin B 1 is detrimental to several Physiological processes. In addition to the effect on seed germination and seedling growth, the syntheses of chlorophyll and protein are also affected by the toxin. It appears that deleterious effect of aflatoxin starts with the imbibition of water mixed toxin by the seed because the first effect is visible on seed germination and seedling growth. The enzymatic activity is therefore bound to be disturbed which ultimately affects other physiological activities like chlorophyll and protein syntheses in the present context.

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